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<p>(54) Title: <b>GRANULE WITH HYDRATED BARRIER MATERIAL</b></p> <p>(57) Abstract</p> <p>A granule having high stability and low dust is described. The granule includes a hydrated barrier material having moderate or high water activity. Also described are methods of producing the granules.</p>			

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## GRANULE WITH HYDRATED BARRIER MATERIAL

### Background of the Invention

5 Recently the use of enzymes, especially of microbial origin, has become more and more common. Enzymes are used in several industries including, for example, the starch industry, the dairy industry, and the detergent industry. It is well known in the detergent industry that the use of enzymes, particularly proteolytic enzymes, has created industrial hygiene concerns for detergent factory workers, particularly due to the health risks  
10 associated with dustiness of the available enzymes.

Since the introduction of enzymes into the detergent business, many developments in the granulation and coating of enzymes have been offered by the industry. See for example the following patents relating to enzyme granulation:

U.S. Patent 4,106,991 describes an improved formation of enzyme granules by  
15 including within the composition undergoing granulation, finely divided cellulose fibers in an amount of 2-40% w/w based on the dry weight of the whole composition. In addition, this patent describes that waxy substances can be used to coat the particles of the granulate.

U.S. Patent 4,689,297 describes enzyme containing particles which comprise a particulate, water dispersible core which is 150 - 2,000 microns in its longest dimension, a  
20 uniform layer of enzyme around the core particle which amounts to 10%-35% by weight of the weight of the core particle, and a layer of macro-molecular, film-forming, water soluble or dispersible coating agent uniformly surrounding the enzyme layer wherein the combination of enzyme and coating agent is from 25-55% of the weight of the core particle. The core material described in this patent includes clay, a sugar crystal enclosed in layers of corn  
25 starch which is coated with a layer of dextrin, agglomerated potato starch, particulate salt, agglomerated trisodium citrate, pan crystallized NaCl flakes, bentonite granules or prills, granules containing bentonite, Kaolin and diatomaceous earth or sodium citrate crystals. The film forming material may be a fatty acid ester, an alkoxylated alcohol, a polyvinyl alcohol or an ethoxylated alkylphenol.

30 U.S. Patent 4,740,469 describes an enzyme granular composition consisting essentially of from 1-35% by weight of an enzyme and from 0.5-30% by weight of a synthetic fibrous material having an average length of from 100-500 micron and a fineness in the range of from 0.05-0.7 denier, with the balance being an extender or filler. The granular composition may further comprise a molten waxy material, such as polyethylene  
35 glycol, and optionally a colorant such as titanium dioxide.

U.S. Patent 5,254,283 describes a particulate material which has been coated with a continuous layer of a non-water soluble, warp size polymer. U.S. Patent 5,324,649

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describes enzyme-containing granules having a core, an enzyme layer and an outer coating layer. The enzyme layer and, optionally, the core and outer coating layer contain a vinyl polymer.

WO 91/09941 describes an enzyme containing preparation whereby at least 50% of the enzymatic activity is present in the preparation as enzyme crystals. The preparation can be either a slurry or a granulate.

WO 97/12958 discloses a microgranular enzyme composition. The granules are made by fluid-bed agglomeration which results in granules with numerous carrier or seed particles coated with enzyme and bound together by a binder.

However, even in light of these developments offered by the industry (as described above) there is a continuing need for low-dust enzyme granules which have additional beneficial characteristics. Additional beneficial characteristics needed in the enzyme granulation industry are low-residue granule formulations (where low residue is defined as a reduced tendency to leave noticeable undissolved residues on clothes or other material), and improved stability formulations. Accomplishing all these desired characteristics simultaneously is a particularly challenging task since, for example, many delayed release or low-dust agents such as fibrous cellulose or warp size polymers leave behind insoluble residues.

Therefore, it is an object of the present invention to provide low-dust, low residue, highly soluble enzyme granules having increased stability. It is another object of the present invention to provide processes which afford the formation of such improved granules.

#### Summary of the Invention

One embodiment of the present invention is a granule that includes a protein core and a hydrated barrier material with moderate or high water activity. The hydrated barrier material can be in one or more layers and/or can be included in the protein core.

A further embodiment of the present invention is a granule that includes an enzyme core and a hydrated barrier material with moderate or high water activity. The hydrated barrier material can be in one or more layers and/or can be included in the enzyme core.

Another embodiment is a method of producing the above granule.

#### Detailed Description of the Invention

The present invention provides a granule with improved stability having low dust. The granule includes a protein core and a hydrated barrier material with moderate or high water activity.

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A "protein core" or an "enzyme core" can be homogenous such as that described in US patent application 08/995,457 or layered as described in U. S. Pat. No. 5,324,649.

Proteins that are within the scope of the present invention include pharmaceutically important proteins such as hormones or other therapeutic proteins and industrially important proteins such as enzymes.

Any enzyme or combination of enzymes may be used in the present invention. Preferred enzymes include those enzymes capable of hydrolyzing substrates, e.g. stains. These enzymes are known as hydrolases which include, but are not limited to, proteases (bacterial, fungal, acid, neutral or alkaline), amylases (alpha or beta), lipases, cellulases and mixtures thereof. Particularly preferred enzymes are subtilisins and cellulases. Most preferred are subtilisins such as described in U.S. Patent 4,760,025, EP Patent 130 756 B1 and EP Patent Application WO 91/06637, which are incorporated herein by reference, and cellulases such as Multifect L250™ and Puradax™, commercially available from Genencor International. Other enzymes that can be used in the present invention include oxidases, transferases, dehydratases, reductases, hemicellulases and isomerases.

As noted, the barrier material can be coated over the protein core in one or more layers or made part of the protein core in order to insulate or to impede transport of water and inactivating substances to the protein. When the barrier material is part of the protein core, it can be dispersed throughout the core or as a layer in the core.

Suitable hydrated barrier materials with moderate or high water activity can include salts of an inorganic or organic acid, sugars, polysaccharides, lipids, proteins or synthetic polymers; preferably salts.

The term "water activity", symbolized  $a_w$ , refers to the fractional relative humidity of an atmosphere in equilibrium with a solid or liquid phase material, i.e., the ratio of the partial pressure of water vapor to that present above pure water at the same temperature. In all phases between which water distribution has reached equilibrium, it is by definition equal. The term "relative humidity" is generally used to describe the water in the atmosphere or gas phase in equilibrium with the solid, and is expressed as a percentage, with 100% as the relative humidity of pure water in a closed system. Thus, for any water activity value, there is a corresponding relative humidity given by  $\%RH = 100 * a_w$ .

Water activity can be readily measured by methods known in the art, typically by placing a sample of the material inside the temperature-controlled chamber of a water activity meter, such as the Water Activity System Model D2100 available from Rotronic Instrument Corp. (Huntington, NY), and allowing the measurement to reach equilibrium as indicated on the display.

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A "hydrated" barrier material contains water in a free or bound form, or a combination of the two. The water of hydration can be added either during or after the coating process. The degree of hydration will be a function of the material itself and the temperature, humidity and drying conditions under which it is applied.

5 "Moderate or high" water activity includes a water activity of at least 0.25, preferably greater than 0.30, most preferably greater than 0.35. The water activity referred to herein is that of the granule itself once it has the barrier material--but no further coatings--coated onto it. Further coatings may mask accurate measurement of the water activity of the barrier material as a distinct layer.

10 Without wishing to be bound by theory, it is expected that materials with a water activity greater than 0.25 will have a reduced driving force for picking up water under storage conditions in which the relative humidity is greater than 25%. Most climates have relative humidities above 25%. Many detergents have water activities in the range of about 0.3 to 0.4. If the water activity of the granule is actually higher than that of the surrounding  
15 detergent or storage climate, the driving force for pick up of water by the granule should be eliminated, and in fact water may be given up by the granule to its surroundings. Even if the water activity of the granule is lower than that of the detergent or the corresponding relative humidity, the water present in the barrier layer would act as a shield limiting the amount of water and hence in activating substances being picked up by the granule and affecting the  
20 protein core.

In the case of salt hydrates, the hydrated material is a crystalline salt hydrate with bound water(s) of crystallization. The hydrate should be chosen and applied in a manner such that the resulting coated granule will have a water activity in excess of 0.25, or as high as possible while still providing a granule which is dry to the touch. By applying a salt  
25 hydrate, or any other suitable hydrated barrier material, in such a manner, as noted above, one expects that this would eliminate any driving force for further uptake of water by the granule. As an important consequence, the driving force for transport of substances which may be detrimental to enzyme activity, such as perborate or peroxide anion, is removed. Without water as a vehicle, these substances are less likely to penetrate the enzyme core.  
30 Empirical data demonstrates that enzyme activity in the granule is substantially enhanced by coating the enzyme core with stable salt hydrates.

Preferred salts include magnesium sulfate heptahydrate, zinc sulfate heptahydrate, copper sulfate pentahydrate, sodium phosphate dibasic heptahydrate, magnesium nitrate hexahydrate, sodium borate decahydrate, sodium citrate dihydrate and magnesium acetate  
35 tetrahydrate.

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The granules of the present invention can also comprise one or more coating layers. For example, such coating layers may be one or more intermediate coating layers, or such coating layers may be one or more outside coating layers or a combination thereof. Coating layers may serve any of a number of functions in a granule composition, depending on the end use of the granule. For example, coatings may render the protein resistant to oxidation by bleach, bring about the desirable rates of dissolution upon introduction of the granule into an aqueous medium, or provide a barrier against ambient moisture in order to enhance the storage stability of the enzyme and reduce the possibility of microbial growth within the granule.

Suitable coatings include polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), cellulose derivatives such as methylcellulose, hydroxypropylmethyl cellulose, hydroxycellulose, ethylcellulose, carboxymethyl cellulose, hydroxypropyl cellulose, polyethylene glycol, polyethylene oxide, chitosan, gum arabic, xanthan, carrageenan, latex polymers, and enteric coatings. Furthermore, coating agents may be used in conjunction with other active agents of the same or different categories.

Suitable PVAs for incorporation in the coating layer(s) of the granule include partially hydrolyzed, fully hydrolyzed and intermediately hydrolyzed PVAs having low to high degrees of viscosity. Preferably, the outer coating layer comprises partially hydrolyzed PVA having low viscosity. Other vinyl polymers which may be useful include polyvinyl acetate and polyvinyl pyrrolidone. Useful copolymers include, for example, PVA-methylmethacrylate copolymer and PVP-PVA copolymer.

The coating layers of the present invention may further comprise one or more of the following: plasticizers, extenders, lubricants, pigments, and optionally additional enzymes. Suitable plasticizers useful in the coating layers of the present invention are plasticizers including, for example, polyols such as sugars, sugar alcohols, or polyethylene glycols (PEGs), urea, glycol, propylene glycol or other known plasticizers such as triethyl citrate, dibutyl or dimethyl phthalate or water. Suitable pigments useful in the coating layers of the present invention include, but are not limited to, finely divided whiteners such as titanium dioxide or calcium carbonate or colored pigments or dyes or a combination thereof. Preferably such pigments are low residue pigments upon dissolution. Suitable extenders include sugars such as sucrose or starch hydrolysates such as maltodextrin and corn syrup solids, clays such as kaolin and bentonite, and talc. Suitable lubricants include nonionic surfactants such as Neodol, tallow alcohols, fatty acids, fatty acid salts such as magnesium stearate and fatty acid esters.

The outer coating layer of the present invention preferably comprises between about 1-25% by weight of the coated granule.

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Adjunct ingredients may be added to the granules of the present invention. Adjunct ingredients may include: metallic salts; solubilizers; activators; antioxidants; dyes; inhibitors; binders; fragrances; enzyme protecting agents/scavengers such as ammonium sulfate, ammonium citrate, urea, guanidine hydrochloride, guanidine carbonate, guanidine sulfamate, thiourea dioxide, monoethanolamine, diethanolamine, triethanolamine, amino acids such as glycine, sodium glutamate and the like, proteins such as bovine serum albumin, casein and the like etc.; surfactants including anionic surfactants, ampholytic surfactants, nonionic surfactants, cationic surfactants and long-chain fatty acid salts; builders; alkalis or inorganic electrolytes; bleaching agents; bluing agents and fluorescent dyes and whiteners; and caking inhibitors.

The granules described herein may be made by methods known to those skilled in the art of enzyme granulation, including pan-coating, fluid-bed coating, fluid-bed agglomeration, prilling, disc granulation, spray drying, extrusion, centrifugal extrusion, spherization, drum granulation, high shear agglomeration, or combinations of these techniques.

The following examples are representative and not intended to be limiting. One skilled in the art could choose other proteins, protein cores, enzymes, enzyme cores, seed particles, methods and coating agents based on the teachings herein.

20 **Examples**

**Example 1. Stability of Magnesium Sulfate Coated Protease Granules**

A. In a Deseret 60 fluidized bed coater, 54.1 kg of sucrose/starch non pareil seeds were charged and fluidized. Onto these cores, 75.8 kg of protease UF concentrate containing 62.9 g/kg subtilisin protease were sprayed under the following conditions.  
25 (Ranges indicate initial and final values over the course of the specified ramp time):

Ramp time:	80 minutes
Fluid feed rate	0.6-1.0 liter/min
Atomization pressure	75 psi
30 Inlet air temperature	85-92 degrees C
Outlet air temperature	50 degrees C
Fluidization air rate	18 m <sup>3</sup> /min

A solution of magnésium sulfate was prepared by adding 22.2 kg of magnesium sulfate heptahydrate into 22.2 kg of water, and this was sprayed onto the enzyme-coated cores under the following conditions in order to provide that 20% of the final granule would

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be magnesium sulfate heptahydrate, with care being taken to keep the bed temperature close to, but slightly below, 50 degrees C:

5           Ramp time:                   40 minutes  
Fluid feed rate                       0.6-1.7 liter/min  
Atomization pressure               45 psi  
Inlet air temperature              70-84 degrees C  
Outlet air temperature             48-50 degrees C  
Fluidization air rate             18 m3/min

10

Finally, a polymer coating solution was prepared by dissolving 6.35 kg of Elvanol 51-05 polyvinyl alcohol, 7.94 kg titanium dioxide and 1.59 kg Neodol 23-6.5T nonionic surfactant in 50.12 kg water and spraying over the salt-coated enzyme cores under the following conditions:

15

Ramp time:                   10 min, then constant for 100 min  
Fluid feed rate               0.6 liter/min  
Atomization pressure       75 psi  
Inlet air temperature       50 degrees C  
20           Outlet air temperature   75-80 degrees C  
Fluidization air rate       18 m3/min

The harvested granules had an enzyme concentration of approximately 40 g/kg.

25           B. Accelerated Stability Test

The stability of many enzyme granules formulated into bleach-containing detergents is generally excellent, showing generally no more than about 10 to 20% loss in activity over 6 weeks storage at 30 to 37° C and 70% to 80% R.H. However, to aid in the development and screening of granular formulations, it is desirable to have an accelerated means of 30 determining relative granule stability. The conditions of the accelerated stability test (AST) are far more severe than enzyme granules or detergents would ever encounter in realistic storage or transport. The AST is a "stress test" designed to discriminate differences between formulations which would otherwise not be evident for weeks or months.

In this test, a test detergent base was made from the following ingredients:

35

72% WFK-1 detergent base

(WFK, Forschungsinstitut fuer  
Reinigungstechnologie e.V.,

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Krefeld, Germany).

25%	sodium perborate monohydrate	(Degussa Corp., Allendale Park, New Jersey.)
5	3% TAED bleach activator (= tetraacetyl ethylenediamine)	(Warwick International, Mostyn, UK)

For each enzyme sample to be tested, three identical tubes were prepared by  
10 adding 1 gram of the test base and 30 mg of enzyme granules to a 15 ml conical tube and  
mixed by inverting the capped tube 5-8 times by hand. A hole was drilled in the tube cap  
with a 1/16 inch drill bit. One of the three tubes was assayed immediately and the other two  
were stored in a humidity chamber set at 50° C and 70% R.H. One of the two stored tubes  
was assayed after 1 day of storage; the second, after 3 days of storage. Storage stability  
15 was reported for Day 1 and Day 3 by dividing the remaining activity by the original activity at  
Day 0, expressed as a percentage.

The enzyme activity was determined by adding to each tube 30 ml of 0.25M MES pH  
5.5 buffer containing 20 µl Catalase HP L5000 (Genencor International, Rochester, NY) and  
incubating for 40 minutes to inactivate the perborate. After this, the enzyme was assayed  
20 by adding 10 µl of the test tube mixture and 10 µl of sAAPF protease substrate to 980 µl of  
0.1M Tris pH 8.6, then incubating at 25°C over 3 minutes, and measuring the optical  
absorbance at 410 nm. The slope of the absorbance vs. time was then multiplied by the  
dilution factor and the known extinction coefficient for the specific protease to obtain an  
enzyme activity as concentration in mg/ml.

25 The process described in A above was repeated three more times, the only  
difference being that the outlet air temperature was controlled at a setpoint of 40, 60 and 70  
degrees C in each of the three separate runs. Samples were removed from all four batches  
after the magnesium sulfate barrier coating had been applied, and water activities of the  
granules were measured in a Rotronic Water Activity System, as reported in Table 1. Two of  
30 the granules, after application of the final polymer coating, were placed in WFK-1 detergent  
formula and stored in tubes with drilled caps for three days at 50 degrees C and 70%  
relative humidity, according to the accelerated stability test method described above. Tubes  
were withdrawn from the humidity chamber and assayed after 1 day and 3 days. The  
percent retained activities are reported in Table 1. The results indicate the granules in  
35 which magnesium sulfate was coated at 50 degrees C outlet temperature are significantly  
more stable than those coated at 70 degrees C, and that the more stable granules had a  
water activity above 0.35, while the less stable granules had a significantly lower water  
activity.

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**Table 1. Stability of Magnesium Sulfate Coated Enzyme Granules**

Outlet Temp (C)	A <sub>w</sub> of MgSO <sub>4</sub> Coated Protease Cores	Percent Retained Activity of Granules Stored in Bleach Detergent			
		0 days	1 day	3 days	6 months
40	0.374				
50	0.409	100%	108%	97%	
60	0.140				
70	0.165	100%	94%	63%	

**Example 2. Stability of Sodium Citrate Coated Protease Granules**

5 A. In a Vector 60 coater, 25 kg of sucrose/starch nonpareil seeds were fluidized and 30.9 kg of subtilisin protease concentrate with a concentration of 65.9 g/L and 18.3% total solids were sprayed onto the fluidized cores under the following conditions:

10      Ramp time:                55 minutes  
         Fluid feed rate            0.5-0.9 liter/min  
         Atomization pressure    75 psi  
         Inlet air temperature    60-95 degrees C  
         Outlet air temperature   50 degrees C  
         Fluidization air rate    24 m<sup>3</sup>/min

15      A solution of trisodium citrate was prepared by adding 13.2 kg of trisodium citrate dihydrate into 19.7 kg of water, and this was sprayed onto the enzyme-coated cores under the following conditions in order to provide that 25% of the final granule would be trisodium citrate dihydrate, with care being taken to keep the bed temperature close to 50 degrees C:

20      Ramp time:                23 minutes  
         Fluid feed rate            0.6-1.9 liter/min  
         Atomization pressure    75 psi  
         Inlet air temperature    60-95 degrees C  
         Outlet air temperature   50 degrees C  
         Fluidization air rate    24 m<sup>3</sup>/min

Finally, a polymer coating solution was prepared by dissolving 2.94 kg Methocel HPMC, 0.98 kg polyethylene glycol, molecular weight 600, 2.06 kg titanium dioxide and 0.59 kg

-- 10 --

Neodol 23-6.5T nonionic surfactant in 55.88 kg water and spraying over the salt-coated enzyme cores under the following conditions:

Ramp time: 10 min, then 80 minutes constant  
 5 Fluid feed rate 0.5 - 0.7 liter/min  
 Atomization pressure 75 psi  
 Inlet air temperature 75-80 degrees C  
 Outlet air temperature 60 degrees C  
 Fluidization air rate 18 m<sup>3</sup>/min

10

The harvested granules had a weight of 49.5 kg and an enzyme concentration of approximately 40 g/kg.

B. The above process was repeated under the same conditions, but the outlet air  
 15 temperature was controlled at a setpoint of 70 degrees C. Samples were removed from both batches after the sodium citrate barrier coating had been applied, and water activities of the granules were measured in a Rotronic Water Activity System, as reported in Table 2. The two granules, after application of the final polymer coating, were placed in an automatic dish detergent base and stored in sealed tubes for 84 days at 37 degrees C. Tubes were  
 20 withdrawn from the humidity chamber and assayed after 14, 42 and 84 days. The percent retained activities are reported in Table 2. The results indicate the granules in which sodium citrate was coated at 50 degrees C outlet temperature are significantly more stable than those coated at 70 degrees C, and that the more stable granules had a water activity above 0.25, while the less stable granules had a significantly lower water activity.

25

**Table 2. Stability of Sodium Citrate Coated Enzyme Granules**

Outlet Temp (C)	$A_w$ of Na <sub>3</sub> Citrate Coated Protease Cores	Percent Retained Activity of Granules Stored in Bleach Detergent			
		0 days	14 days	42 days	84 days
55	0.272	100%	90%	89%	87%
70	0.059	100%	86%	81%	75%

**Claims**

1. A granule comprising an enzyme core and a barrier material, wherein the barrier material comprises a hydrated barrier material with moderate or high water activity.
2. The granule of claim 1, wherein the barrier material is a salt.
3. The granule of claim 1, wherein the salt is selected from the group consisting of magnesium sulfate heptahydrate, zinc sulfate heptahydrate, copper sulfate pentahydrate, sodium phosphate dibasic heptahydrate, magnesium nitrate hexahydrate, sodium borate decahydrate, sodium citrate dihydrate and magnesium acetate tetrahydrate.
4. The granule of claim 1, wherein the barrier material is part of the protein core.
5. The granule of claim 1, wherein the barrier material is coated over the protein core.
6. The granule of claim 1, further comprising a layer of material between the protein core and the barrier material.
7. The granule of claim 1, further comprising a layer of material over the barrier layer and protein core.
8. The granule of claim 1, wherein the protein is an enzyme.
9. The granule of claim 1, wherein the water activity is greater than 0.25.
10. A method of producing a granule comprising:
  - a) providing a protein core;
  - b) coating a hydrated barrier material with moderate or high water activity onto the protein core.
11. The method of claim 10, further comprising a coating over the barrier material.

# INTERNATIONAL SEARCH REPORT

Inte      ional Application No  
PCT/US 98/27214

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6    C11D3/386    C11D3/38

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6    C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 43 44 215 A (COGNIS GESELLSCHAFT FÜR BIO- UND UMWELTTECHNOLOGIE) 29 June 1995 see column 7, last paragraph - column 8, paragraph 2; example 1 ---	1,4,8,10
X	DE 40 41 752 A (HENKEL) 25 June 1992 see page 3, paragraph 5; claims 1,12 ---	1,4,8,10
A	WO 97 12958 A (GENENCOR INTERNATIONAL) 10 April 1997 see page 4, paragraph 2; claims 1,3 ---	1,2,8,10
P,A	WO 98 45395 A (PROCTER & GAMBLE) 15 October 1998 see claims 1,7; example 1 ---	1-4,8,10 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 May 1999	04/06/1999
Name and mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  Van Bellingen, I

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International Application No  
PCT/US 98/27214

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